Asmarines A−F, Novel Cytotoxic Compounds from the Marine Sponge *Raspailia* Species

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Three pairs of nitrogen-containing metabolites, asmarines A−F (**1**−**6**), were isolated from the Red Sea sponge *Raspailia* sp., collected in the Dahlak Archipelago, Eritrea. Although the first pair could fully be separated to give compounds **1** and **2**, the other two pairs could only be enriched up to about 80% of one isomer. The structures of the new compounds were established by spectroscopic means. Besides the asmarines, methyl 3-oxo-cholan-24-oate (**12**) was also isolated. The absolute configuration of asmarine A (**1**) was determined on the basis of CD measurements of its unstable 18-oxo derivative (**7**) and mainly the Cotton effect of the dicarbonyl derivative (**9**) of chelodane (**8**). A *O,N*(7′)-dimethyl derivative (**10**) and a second, unexpected, methylated product (**11**) were obtained from **2**.

In our continued search for biologically active metabolites from Red Sea marine invertebrates,[1−3] we found that the EtOAc extract of a marine sponge *Raspailia* sp., collected near Nakora island, Dahlak Archipelago, Eritrea, was cytotoxic to four human cancer cell lines.[4] Bioassay-guided fractionation resulted in the isolation of two novel nitrogen-containing metabolites, asmarines A and B (**1** and **2**).[4] The structure of asmarine A was ascertained by an X-ray diffraction analysis.[4]

Results and Discussion

Asmarines A and B (Figure 1) represent a new class of nitrogen-containing metabolites. Biosynthetically, the purine portion of the molecules originates, most likely, from adenine, while the other 20 carbon atoms come from a diterpene, chelodane (**8**).[5] Chelodane and zaatirin[4,5] were also isolated from the sponge together with the asmarines. A similar biogenesis leads to the agelasines.[6] However, in the case of the asmarines, the vinyl carbinol moiety of chelodane closes a third heterocycle, namely, a diazacycloheptane. The ^{15}N-chemical shifts of the various nitrogen atoms of **1** were measured from an ^{15}NH−HMBC experiment[7] and were found to be δ_N 163.4, 130.1 (N-7′,-9′), 160.0, 152.0 (N-1′,-3′), and 134.7 (NOH) ppm, when compared with $HCONH_2$, δ_N 112.0 ppm, as a standard.

To determine the absolute configuration of **1** after the relative configuration was assigned by the X-ray analysis, its ozonloysis, aimed to afford the 18-oxo derivative for CD measurements, was undertaken. Under mild conditions (ozone for 30 s in MeOH, −78 °C) asmarine A afforded the expected 18-oxo derivative (**7**) leaving the heterocyclic system intact. The structure of **7** ($[\alpha]_D$ +32°, *m/z* 426) was determined from its spectral data, mainly the NMR resonances, namely, the disappearance of the external methylene (δ_C 102.5 t and δ_H 4.60 s) and the appearance of a new carbonyl group at δ_C 215.0 ppm. Unfortunately, compound **7** was not stable, most likely due to base-autocatalyzed decomposition. Nevertheless, a weak positive Cotton effect could be observed in the 280−300 nm region. It was, however, accompanied by additional effects. Asmarine A by itself does not show any Cotton effect in this region. Although from the weak positive effect the absolute configuration could be suggested, we preferred to prepare the corresponding ozonolysis product of chelodane, which is, most likely, the biogenetic precursor of **1**.

On the basis of the absolute configuration of chelodane, (see below) and accepting the above-suggested biogenesis that chelodane is the precursor of the diterpene part of **1**, the 5*R*,8*R*,9*S*,10*R*,13*S* absolute configuration is suggested for asmarine A and, tentatively, assuming a similar biosynthesis, for all six asmarines (Figure 1).

The absolute configuration of chelodane (**8**) was determined from the optical activity of its ozonolysis product **9** (Figure 2). The CD spectrum of compound **9**, the 4,14-dioxo derivative of chelodane, showed a positive Cotton effect, $\Delta\epsilon = +0.36$ (λ 295 nm, CH_3OH) suggesting the 5*R*,8*R*,9*S*,10*R*,13*S* absolute configuration (Figure 2). The latter conclusion is based on the assumption that the perturbation of the 4-carbonyl $n \rightarrow \pi^*$ transition, by the axial neighbor 5-methyl group, is the major one determining the positive sign of the Cotton effect according to the Octant rule.[8]

Asmarine B (**2**), the second major asmarine, possesses the same heterocyclic system as **1** (Tables 1 and 2) but differs in the Decalin portion of the molecule. From the COSY, HMQC, and HMBC experiments (Figure 4 and Table 1), it was evident that the planar structure of the diterpene part of **2** is identical with that of **1**. Changes in the H and C atoms' chemical shifts, however, required a change in the stereochemistry, which was clarified by difference NOE experiments. From the measured NOEs, between Me-19 and H-10 (2.7%) and between H-18 and Me-20 (0.8%), it became clear that **2** possessed a cis rather than a trans Decalin system. Moreover, the NOEs also determined the stereochemistry of the other two chiral centers of the Decalin (see Figure 3 for the key NOEs).[9] The carbon chemical shifts of the suggested substituted cis Decalin system of **2** are essentially identical with those of the corresponding C-atoms in popolohuanone E[10] (Table 2) and arenarol;[11] the different value for C-9 is expected due to the different substitution of this center.

Together with the major two asmarines (**1** and **2**), we have isolated from the sponge extract, in smaller amounts, two additional inseparable pairs of compounds, designated asmarines C and D and asmarines E and F (compounds **3**−**6**), respectively. Tedious chromatographies resulted only in enrichment, in each mixture, of one isomer (up to 80−90%). The ratio between the two counterparts, in each pair,

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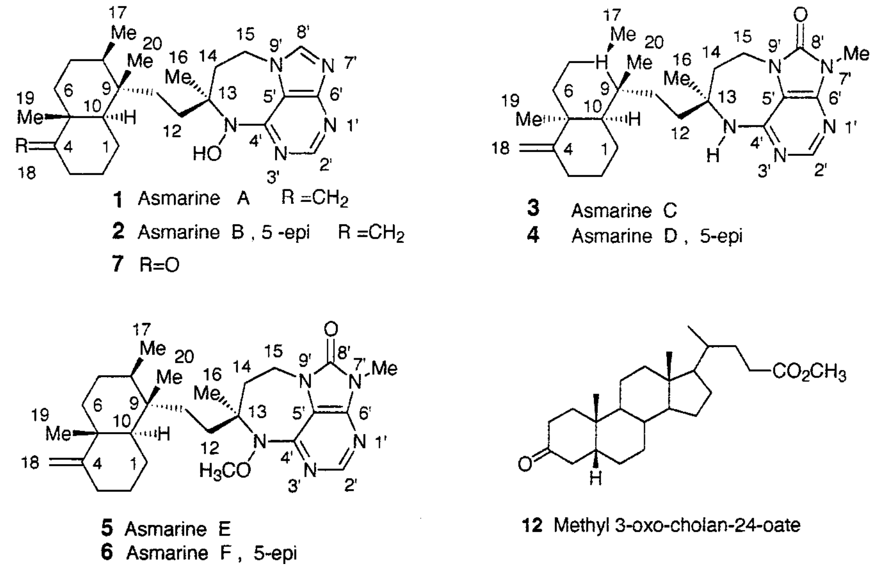


Figure 1. Asmarines A−F and methyl 3-oxo-cholan-24-oate.

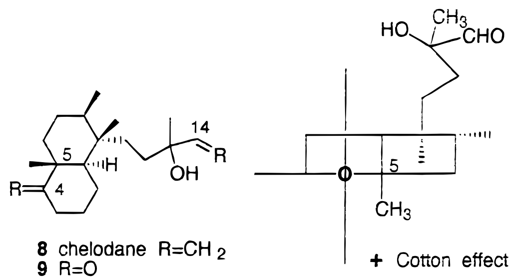


Figure 2. Chelodane and ozonolysis product.

Table 1. ^{1}H NMR of Asmarines A and B (500 MHz, $CDCl_3$)

number	1	J (Hz)	2 J (Hz)	HMBC (2) (H to C)
1	1.70 br d	12.5	1.82 m	2, 9, 10
	1.45 m[a]		1.50 m	3, 9
2	1.85 br d	12.5	1.75 m	1, 3, 4
	1.21 br td	12.0, 3.0	1.60 m	1, 3, 4
3	2.25 dt	13.0, 5.0	2.45 td (13.5, 6.5)	2, 4, 18
	2.05 dd	13.0, 3.0	2.12 m	1, 2, 4, 18
6	1.57 m[a]		2.10 m	7, 8, 10, 19
	1.47 m[a]		1.20 td (13.0, 3.5)	4, 5, 7, 10, 19
7	1.45 m[a]		1.54 m	6, 8, 9
			1.20 m	6, 8, 9
8	1.37 m		1.35 m	7, 9, 17, 20
10	1.05 dd	12.0, 2.0	1.39 m	1, 4, 5, 19, 20
11	1.55 dt	3.0, 12.0	1.59 m	9, 12, 20
	1.25 dt	3.0, 12.0	1.30 dt (2.5, 12.0)	9, 10, 12, 20
12	1.95 dt	4.0, 13.0	1.95 dt (4.0, 13.0)	11, 13, 14, 20
	1.43 m		1.55 m	13, 20
14	2.50 ddd		2.55 m (8 lines)[b]	13, 15, 16
	2.15 dd		2.25 m (7 lines)[b]	12, 13, 15
15	4.25 dt		4.30 br s, 2H	13, 14, 5′, 8′
	4.20 dd			
16	1.44 s		1.49 s, 3H	12, 13, 14
17	0.70 d	6.6	0.74 d, 3H (6.7)	7, 8, 9
18	4.60 s		4.70 br s, 2H (4.0)	3, 4, 5
19	1.00 s		1.11 s, 3H	4, 5, 6, 10
20	0.65 s		0.82 s, 3H	8, 10, 11
2′	8.50 s		8.50 s	4′, 5′, 6′
8′	7.95 s		7.95 s	4′, 5′, 6′

[a] Overlapping of seven proton signals (H-1, H_2-6, H_2-7 and H-12). [b] Second-order signal.

was determined by the ratio of the H_2-18 and the various methyls' signals in the proton NMR spectrum (Table 3) and mainly by the ratio of the carbon atom lines in the ^{13}C NMR spectrum (Table 2). The ^{13}C resonances, namely comparisons with **1**, **2**, chelodane,[5] and popolohuanone E,[10] suggested that the difference between compounds **3** and **4** and between **5** and **6** is in the ring junction of the Decalin part, as in the case of asmarines A and B (Table 2).

Compounds **3**−**6** differ from **1** and **2** in the substitution pattern of the heterocyclic system. All four compounds lack the 8′-proton, which in **1** and **2** had CH-correlations to C-5′ and -6′. Instead, C-8 is now a carbonyl group resonating at 151−152 ppm (ν_{max} 1690 cm^{-1}) as in other known 8-oxopurines.[12,13] In addition, N(7′) carries a methyl group (δ_H 3.50 and δ_C 26.8 ppm) whose position was determined from its CH correlations to C-6′ and -8′ (Figure 4).[14] The other CH correlations, seen in the HMBC experiment, agree unambiguously with the suggested structure (Figure 1). The molecular peak in the mass spectrum of compounds **3** and **4**, $C_{26}H_{39}N_5O$, is higher by 14 mu than that of **1** and **2**, pointing to the replacement of a proton by a methyl group. The above-discussed change of the imidazole ring of **1** and **2** to an *N*(7′)-methyl-8-oxo-imidazole ring, in **3** and **4**, accounts for the extra methyl group and requires that the hydroxylamine of **1** and **2** lose its oxygen atom in favor of C-8 (Figure 1). The replacement of the hydroxylamine group by a secondary amine (its biogenetic precursor) caused an upfield shift of about 7 ppm in both vicinal C-4′ and C-13 atoms. Indeed, the NH group was observed in the NMR spectrum, taken in DMSO-d_6, at δ_H 7.35, and it demonstrated a NOE between the NH and CH_3-16. The NH group, being in a neopentyl position and hindered by the pyrimidine ring on the other side was not amidated. Even in the case of the hydroxylamine, the NOH group is still hindered (see below). The structures of **3** and **4** were further supported by the MS fragmentations, that is, the change of the 188 base peak of **1** and **2** into 218 (100%) in the spectra of **3** and **4** (Figure 5).

The last pair of isolated asmarines, E and F, compounds **5** and **6**, respectively, analyzed by HREIMS for $C_{27}H_{41}N_5O_2$. The latter formula, together with the NMR data, pointed, in comparison with compounds **1** and **2**, to an additional *N*-methyl group (δ_C 26.5 q), substitution of the oxime hydroxyl by an OMe function (δ_C 64.8 q), and addition of a carbonyl (δ_C 152.2 s) (together 44 amu). From the ^{13}C chemical shifts (Table 2), it was evident that the Decalin parts of **5** and **6** are identical with those in **1**−**4**, whereas the imidazole ring of **1** and **2** changed into the *N*(7′)-methyl-8′-oxo derivative, as in compounds **3** and **4** (Figure 1). The

Table 2. ^{13}C NMR of Asmarines A–F (**1**–**6**)

number	**1**	c[a]	**2**	p[b]	**3**	**4**	**5**	**6**
1	21.8 t	21.8 t	21.2 t	23.1 t	21.3 t	21.9	22.1 t	21.2
2	28.6 t	28.9 t	24.1 t	25.5 t	24.1 t	28.6	28.4 t	24.0
3	33.2 t	33.2 t	31.7 t	32.3 t	31.5 t	32.8	32.9 t	31.5
4	160.6 s	160.6 s	153.6 s	154.0 s	153.4 s	160.1	160.0 s	153.1
5	40.1 s	40.0 s	39.4 s	39.8 s	39.4 s	40.0	40.0 s	39.3
6	37.2 t	37.2 t	38.1 t	38.0 t	38.1 t	37.1	37.1 t	37.9
7	27.4 t	27.9 t	27.2 t	28.4 t	27.2 t	27.2	27.3 t	27.1
8	36.7 d	36.6 d	38.1 d	39.2 d	38.1 d	36.7	36.3 d	38.0
9	39.3 s	39.2 s	40.5 s	44.9 s	40.5 s	39.3	39.3 d	40.6
10	48.6 d	48.6 d	46.6 d	48.2 s	46.7 d	48.7	48.4 d	46.4
11	31.2 t	32.2 t	31.1 t		31.4 t	31.2	31.8 t	29.6
12	33.0 t	35.6 t	31.6 t		33.7 t	33.0	32.9 t	33.0
13	64.2 s		65.0 s		58.2 s	58.2	66.2 s	66.3
14	36.7 t		36.4 t		37.4 t	37.8	37.1 t	37.0
15	42.3 t		42.3 t		39.7 t	39.6	38.5 t	38.5
16	21.8 q		23.1 q		26.7 q	26.6	26.3 q	24.2
17	15.9 q		15.8 q		15.8 q	15.9	15.8 q	15.6
18	102.5 t		105.7 t		105.9 t	102.8	102.7 t	105.7
19	20.1 q		32.9 q		32.9 q	20.8	20.8 q	32.0
20	18.3 q		19.9 q		19.8 q	18.3	18.3 q	19.9
2′	151.7 d		151.6 d		151.0 d	151.0	151.1 d	151.1
4′	149.0 s		149.6 s		142.5 s	142.5	146.7 s	146.7
5′	109.3 s		109.3 s		102.8 s	104.7	104.7 s	104.7
6′	158.7 s		158.4 s		146.8 s	146.3	148.0 s	148.0
8′	143.1 d		143.3 d		151.9 s	151.9	152.2 s	152.3
NOMe							64.8 q	64.9
N(7′)Me					26.8 q	26.8	26.5 q	26.5

[a] c = chelodane.[5] [b] p = popolohuanone E.[10]

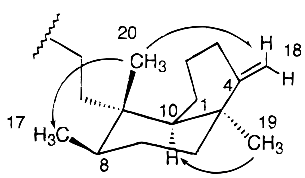


Figure 3. Asmarine B: key NOEs.

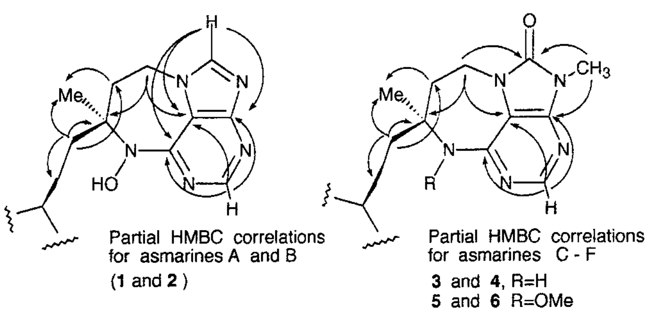


Figure 4. Partial CH correlations.

^{13}C spectrum also established the location of the extra OCH_3 group on the nitrogen atom between carbons 13 and 4′, namely, the existence of a methoxylamine rather than the hydroxylamine group in compounds **1** and **2**. The ^{13}C NMR resonance line of the NOMe group at δ_C 64.8 agrees well with such a group[15] and is similar to the value obtained for this group in derivative **10**, see below. As a result of the NOMe group, both C-13 and C-4′ shifted downfield, in comparison with the corresponding C atoms in compounds **3** and **4**, and became closer to the corresponding values in **1** and **2** (Table 2).

Besides the diterpenes and asmarines, we have also isolated from the sponge methyl 3-oxo-cholan-24-oate (**12**); to the best of our knowledge this is the first reported 5β-cholanic acid derivative from a marine source. The structure was determined from the MS and NMR data (see Experimental Section) and comparison with the literature.[17]

For comparison purposes, we undertook the methylation and acetylation of asmarines A and B. Methylation of asmarine B (or A) with CH_3I in acetone in the presence of 1% K_2CO_3 at room temperature, overnight, gave a dimethyl derivative (**10**) (Scheme 1), *m*/*z* 453, δ_H 4.07 and 4.21 s, 3H each, δ_C 66.0 and 37.1 ppm, respectively. The carbon chemical shift of the former methyl established its location on the NOH group.[15] The NOMe group (δ_C 66.0 q) was unambiguously confirmed by weak NOEs between it and the vicinal H-2′ and CH_3-16. According to the lowfield chemical shift of the second introduced methyl group (δ_H 4.21 s), its position was suggested to be on a quaternary nitrogen atom (δ_C 37.1). CH correlations from this NMe group to C-6′ (δ 150.6) and C-8′ (δ 146.6) placed it on N(7′), a location that was further supported by a weak NOE observed between the *N*(7′)-methyl group and H-2′. The separation and downfield shift of H-15,-15′ [4.53 and 5.00 in comparison to 4.30 (2H) in **2**] suggested the positive charge to be spread over both nitrogen atoms of the imidazole ring. The chemical shifts of H-15,-15′ were confirmed from CH correlations from this pair to C-5′ (109.0 ppm), C-14 (36.7 ppm), and C-13 (69.8 ppm). Supporting evidence for the chemical shift assignments of the purine part came from CH correlations, in the HMBC spectrum of **10**, between H-2′ and C-4′ and -6′, between H-8′ and C-5′ and -6′, and a weak NOE between H-8′ and H-15. Interestingly, the one-bond CH-coupling constants of CH(2′) and CH(8′) in **10** changed to 213 and 217 Hz, respectively, in comparison with 206 Hz for both in **1** and **2**.

A second reaction performed with asmarine B (**2**) was mild acetylation with Ac_2O in MeOH at room temperature overnight, mild conditions under which only nitrogen protons, and not alcohols, acetylate. TLC and NMR analyses of the crude reaction product pointed to a mixture with no $NOCOCH_3$ group but, surprisingly, with a newly introduced OCH_3 group (δ 4.00s). Chromatography of the mixture on deactivated Si gel gave compound **11** (30%), which analyzed for $C_{26}H_{39}N_5O$ by HREIMS. Comparison of the NMR data of **11** with those of the starting material (**2**) showed clearly, as expected, that the Decalin portion

Table 3. Partial ^{1}H NMR Resonances of Clear Key Signals of Asmarines A−F

number	1	2	3	4	5	6
12	1.95 t	1.95 dt	1.60 m, 2H	1.60 m, 2H	1.05, 2H	1.10 m, 2H
	1.43 m	1.55 m				
14	2.50 ddd	2.55 m	2.34 m	2.20 m, 2H	2.30 m, 2H	2.30 m, 2H
	2.15 ddd	2.25 m	2.25 m			
15	4.25 dt	4.30 t, 2H	4.05 m, 2H	3.91 m, 2H	3.95 m	3.95 m
	4.20 dd				3.65 m	3.65 m
16	1.44 s, 3H	1.49 s, 3H	1.51 s, 3H	1.48 s, 3H	1.55 s, 3H	1.58 s, 3H
17	0.70 d, 3H	0.74 d, 3H	0.75 d, 3H	0.78 d, 3H	0.70 d, 3H	0.67 d, 3H
18	4.60 s, 3H	4.70 d, 2H	4.75 d, 2H	5.05 s, 5.08 s	4.48 s, 2H	4.80 s, 2H
19	1.00 s, 3H	1.11 s, 3H	1.15 s, 3H	1.07 s, 3H	1.04 s, 3H	1.10 s, 3H
20	0.65 s, 3H	0.82 s, 3H	0.90 s, 3H	0.83 s, 3H	0.68 s, 3H	0.66 s, 3H
2′	8.50 s	8.50 s	8.15 s	8.13 s	8.50 s, 1H	8.50 s, 1H
8′		7.95 s, 1H				
N(7′)Me			3.51 s, 3H	3.53 s, 3H	3.52 s, 3H	3.54 s, 3H
NOMe					4.00 s, 3H	4.03 s, 3H

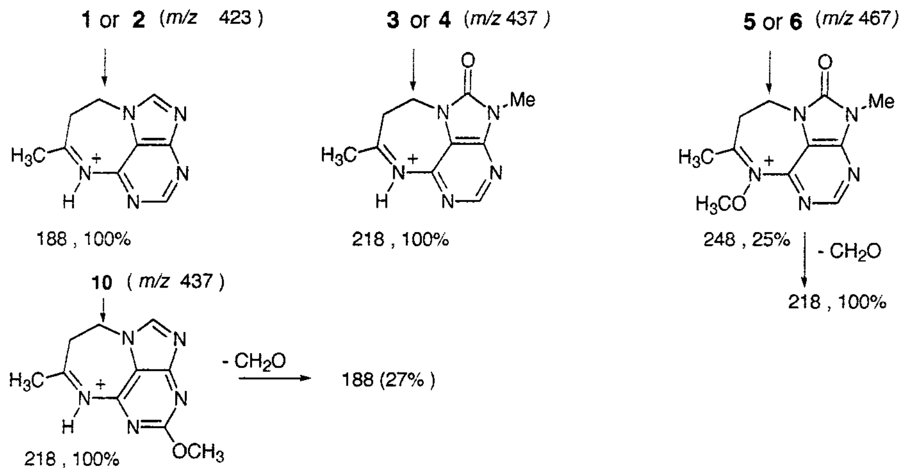
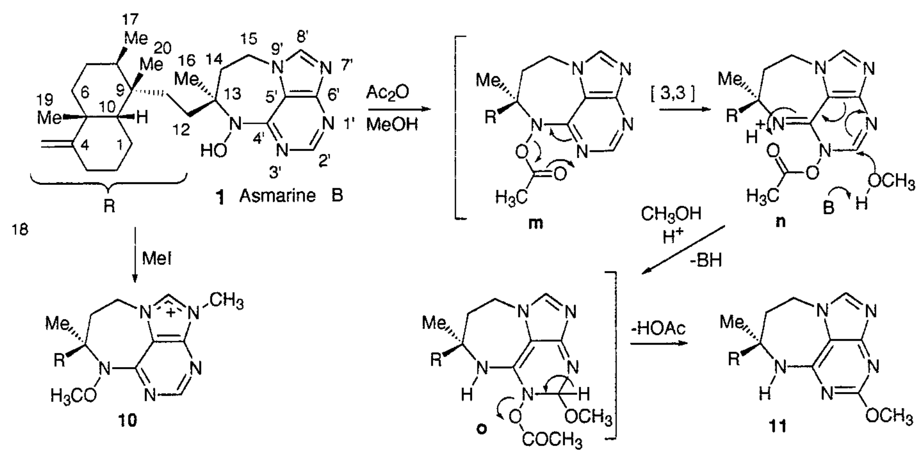


Figure 5. Major MS fragments.

Scheme 1. Reaction Products of Asmarine B



remained intact. The resonance line of C-13, however, moved upfield to 55.5 ppm, resembling the value of this C atom in compounds **3** and **4**. Noticeable was the disappearance of one of the purine protons and the appearance of an OCH_3 group and an acidic proton at 5.50 (br s) ppm (in $CDCl_3$). The chemical shifts of the OCH_3 group, δ_H 4.00 s (3H) and δ_C 54.5q, suggested it to be on an aromatic ring—an assumption that was confirmed by a CH correlation from the OCH_3 protons to δ_C 162.0. Increase in only 14 amu in the molecular weight of **11** required the new oxygen of the OCH_3 group to replace the oxime oxygen, and, therefore, the latter function had to be reduced to a secondary amine. Hence, the broad singlet at δ 5.50 ppm was assigned to this NH group—a suggestion that was confirmed by NOEs between this NH group and H_2-12 and CH_3-16. The suggested structure of **11** (Scheme 1) was further supported from MS fragmentations, as shown in Figure 5.

A suggested four-step mechanism for this rearrangement is shown in Scheme 1. Following the first acetylation step of the NOH group, the newly introduced unstable, spatially hindered group undergoes a [3,3] sigmatropic rearrangement to give intermediate **n**. The third step involves acid-catalyzed 1,6-addition of methanol to give intermediate **o**, which, in the last step, loses acetic acid to give back the aromatic pyrimidine ring.[16]

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet 205 FT−IR spectrophotometer. UV spectrum was obtained on Unikom 931 spectrophotometer.

EIMS or FABMS was recorded on a Fisons Autospect Q instrument. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS (δ_H 0) and $CDCl_3$ (δ_C 77.0).

Animal Material. *Raspailia* sp., subgenus Clathriodendron (class Demospongiae, order Poecilosclerida, family Raspailiidae), is most likely an undescribed species. The sponge is a globular, prickly looking, mustard-colored, slightly lobed sponge that rapidly decomposes underwater into a small anastomosing bush emanating from a single holdfast, with elongated papillae at the branch tips, giving the appearance of being prickly; however, it is soft. The sponge was collected in Nakora, Dahlak Archipelago, Eritrea, the Red Sea, by scuba at a depth of 23 m during May 1997. A voucher sample, sp 25106-ET 310/338, is deposited at the Tel Aviv University museum of zoology.

Extraction and Isolation. The freeze-dried sponge (20 g) was extracted with EtOAc to give, after evaporation, a brown gum (1.2 g). The gum was partitioned between aqueous methanol and *n*-hexane, CCl_4, and $CHCl_3$. The *n*-hexane fraction (550 mg) was chromatographed on Si gel, eluting with hexane−EtOAc with increasing polarity to give chelodane (**8**)[5] (150 mg), zaatirin[4,5] (170 mg), asmarines C and D (**3** and **4**, 5 mg), and asmarines E and F (**5** and **6**, 20 mg). In addition, methyl 3-oxo-cholan-24-oate was also isolated (10 mg).[17] The combined CCl_4, and $CHCl_3$ fractions (240 mg) were chromatographed on a Sephadex LH-20 column eluted with $CHCl_3$−MeOH, 1:1, to give a mixture of asmarines A and B (**1** and **2**, 100 mg). Several crystallizations from methanol and EtOAc−CH_2Cl_2 gave pure crystals of **1**[4] and compound **2**[4] in the mother liquor. The polarities of the various asmarines are as follows: **1** and **2**, R_f = 0.5 (EtOAc−MeOH, 9:1), **3** and **4**, R_f = 0.4 (hexane−EtOAc, 1:1), and **5** and **6**, R_f = 0.55 (hexane−EtOAc, 1:1).

Asmarine A (1):[4] rectangular crystals; mp 232 °C; $[\alpha]_D$ +55° (*c* 0.5, $CHCl_3$); UV λ_{max} (MeOH) 292 nm (ϵ 10 000); EIMS *m/z* 423 (45), 407 (50), 392 (10), 233 (20), 216 (20), 188 (100).

Asmarine B (2):[4] an oil; $[\alpha]_D$ +60° (*c* 0.5, $CHCl_3$); UV λ_{max} (MeOH) 292 nm (ϵ 10 000); FABMS *m/z* 424 (MH^+, 100), 408 (35) 188 (20); HREIMS *m/z* 423.2997, calcd for $C_{25}H_{37}N_5O$, 423.2998.

Asmarines C and D (3 and 4): 1:1 to 4:1 ratio; an oil; IR λ_{max} (neat) 3400 br, 1690, 1632, 1592, 1505, 1467, 1416, 1374 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 437 (60), 218 (100), 422 (5), 246 (5), 218 (100).

Asmarines E and F (5 and 6): 1:1 to 4:1 ratio; crystals from acetone−hexane; mp 160°; IR (neat) ν_{max} 3400 br, 2929, 1721, 1631, 1604, 1505, 1417, 1374, 1250, 1050 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 467 (11), 437 (M − CH_2O; 22), 248 (33), 218 (100), HREIMS *m/z* (calcd) 467.3260 (M^+, 467.3260), 437.3157 (M − CH_2O, 437.3155), 218.1043 ($C_{10}H_{12}N_5O$, 218.1042).

Methyl 3-oxo-cholan-24-oate (12):[17] white crystals; mp 119°; IR(KBr) ν_{max} 2890, 1706, 1250 cm^{-1}; ^{13}C NMR (C_6D_6; 125 MHz) δ 210.0 (s, C-3), 173.5 (s, C-24), 56.1 (d, C-17), 55.9 (d, C-14), 50.7 (q, OCH_3), 44.1 (d, C-5), 42.8 (s, C-13), 42.3 (t, C-4), 40.5 (d, C-9), 40.2 (t, C-12), 37.2 (t, C-2), 36.9 (t, C-1), 35.3 (d, C-20), 35.2 (d, C-8), 34.5 (s, C-10), 31.3 (t, C-23), 31.1 (t, C-22), 28.3 (t, C-16), 26.8 (t, C-7), 25.8 (t, C-6), 24.3 (t, C-15), 22.5 (q, C-19), 21.2 (t, C-11), 18.4 (q, C-21), 12.1 (q, C-18), ^{1}H NMR (C_6D_6, 500 MHz) 3.45 (s, OCH_3), 0.90 (d, Me-21), 0.75 (s, Me-19), 0.60 (s, Me-18); EIMS *m/z* 388 ($C_{25}H_{40}O_3$).

Ozonolysis of Asmarine A (1) to 7. Ozone was passed through a solution of asmarine A (**1**, 10 mg) in CH_2Cl_2 (10 mL) for 30 s. Dimethyl sulfide (0.1 mL) was then added and the solution kept at room temperature overnight. The product, after evaporation of the solvent, was passed through a Sephadex LH-20 column eluted with MeOH−CH_2Cl_2 1:1 to give **7** (4 mg): an oil $[\alpha]_D$ +32° (*c* 0.33, $CHCl_3$); IR (neat) ν_{max} 2966, 2869, 1699, 1654, 1618, 1553 cm^{-1}; ^{13}C NMR ($CDCl_3$) 215.0 s, 158.6 s, 151.7 d, 149.0 s, 143.0 d, 109.3 s, 64.5 s, 48.9 s, 48.2 d, 42.5 t, 42.0 t, 39.6 s, 37.4 t, 37.2 t, 36.0 d, 32.8 t, 31.2 t, 26.4 t, 26.0 t, 22.0 t, 20.7 q, 19.0 q, 18.5 q, 15.8 q; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.33 (br s), 8.14 (br s), 4.35 (br s, H-15), 1.44 (s, Me-16), 1.03 (s, Me-19), 0.71 (d, Me-17), 0.66 (s, Me-20); FABMS *m/z* 426 (MH^+, 65%), 410 (45%), 188 (100%) (see Figure 5).

Ozonolysis of Chelodane (8) to 9. Ozone was passed through a solution of chelodane (**8**, 10 mg) in CH_2Cl_2 (10 mL) for 5 min. Dimethyl sulfide (0.1 mL) was then added and the solution kept at room temperature overnight. The product, after evaporation of the solvent, was filtered through a Si gel column, eluted with hexane−EtOAc to afford the 4,14-dioxo derivative **9**: an oil; $\Delta\epsilon$ +0.36 (295 nm, MeOH); IR ν_{max} (neat) 3460, 2950, 1720, 1450,1050 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 22.6 (t, C-1), 30.7 (t, C-2), 26.2 (t, C-3), 216.3 (s, C-4), 48.9 (s, C-5), 30.1 (t, C-6), 26.5 (t, C-7), 37.4 (d, C-8), 39.4 (s, C-9), 48.5 (d, C-10), 33.0 (t, C-11), 36.0 (t, C-12), 77.6 (s, C-13), 203.6 (t, C-14), 20.6 (q, C-16), 15.9 (q, C-17), 19.1 (q, C-19), 18.7 (q, C-20) (# according to chelodane[4]); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.48 (s, CHO), 3.10 (br s, OH), 2.55 (dt, H-3), 2.20 (dd, H-3′), 2.05 (m, H-2), 1.50 (m, H-2′), 1.30 (s, 3H), 1.12 (s, 3H), 0.80 (d, 3H), 0.78 (s, 3H); EIMS *m/z* 276 (M − H_2O, 5).

Methylation of 2 to 10. A mixture of asmarine B (**2**, 10 mg), MeI (0.2 mL), and 1% aqueous K_2CO_3 (0.1 mL) in acetone (3 mL) was left overnight at room temperature. The mixture was neutralized and evaporated. The residue was partitioned between $CHCl_3$ and H_2O to give, in the organic phase, the *O,N*-dimethyl derivative **10** (5 mg): amorphous powder; IR ν_{max} (neat) 3500, 2927, 1606, 1553, 1451, 1404, 1388, 900 cm^{-1}; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.2 (t, C-1), 24.1 (t, C-2), 31.5 (t, C-3), 153.3 (s, C-4), 39.3 (s, C-5); 38.1 (t, C-6), 27.2 (t, C-7), 38.0 (d, C-8), 40.6 (s, C-9), 46.7 (d, C-10), 30.6 (t, C-11), 31.4 (t, C-12), 69.8 (s, C-13), 36.7 (t, C-14), 43.4 (t, C-15), 24.9 (q, C-16), 15.2 (q, C-17), 105.9 (t, C-18), 32.8 (q, C-19), 19.8 (q, C-20), 146.6 (d, C-2′), 148.7 (s, C-4′), 109.0 (s, C-5′), 150.7 (s, C-6′), 146.7 (d, C-8′), 66.0 (q, OMe), 37.1 (q, NMe); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.72 (s, Me-16), 0.64 (d, Me-17), 4.65 (s, H_2-18), 1.10 (s, Me-19), 0.82 (s, Me-20), 8.68 (s, H-2′), 8.78 (s, H-8′), 4.22 (s, NMe), 4.08 (s, OMe); FABMS *m/z* 453.

Reaction of Asmarine B (2) with Ac_2O−MeOH To Give 11. A solution of asmarine B (**2**, 10 mg) and Ac_2O (0.2 mL) in MeOH (4 mL) was left at room temperature overnight. After evaporation, the residue was chromatographed on a deactivated, MeOH-washed, Si gel column eluted with hexane−EtOAc with increasing polarity to afford compound **11** (3 mg), an oil; IR ν_{max} (neat) 3540, 1610, 1505, 1417, 1374, 1050 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 21.1 (t, C-1), 24.0 (t, C-2), 31.6 (t, C-3), 153.3 (s, C-4), 39.4 (s, C-5); 38.1 (t, C-6), 27.2 (t, C-7), 37.9 (d, C-8), 40.3 (s, C-9), 46.7 (d, C-10), 31.3 (t, C-11), 34.5 (t, C-12), 55.5 (s, C-13), 38.3 (t, C-14), 42.9 (t, C-15), 26.6 (q, Me-16), 15.7 (q, Me-17), 105.9 (t, C-18), 32.9 (q, Me-19), 19.9 (q, C-20), 162.0 (s, C-2′), 148.2 (s, C-4′), 108.0 (s, C-5′), 151.5 (s, C-6′), 143.3 (d, C-8′), 54.5 (q, OCH_3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.35 (dt, H_2-15), 1.39 (s, Me-16), 0.68 (d, Me-17), 4.71 and 4.73 (2 × s, H_2-18), 1.13 (s, Me-19), 0.86 (s, Me-20), 7.86 (s, H-8′), 4.00 (s, Me-2′), 5.50 (br s, N*H*); EIMS *m/z* 437 (45), 422 (15), 407 (10), 246 (10), 218 (100), 188 (25); HREIMS *m/z* 437.3158, calcd for $C_{26}H_{39}N_5O$ 437.3155.

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Supporting Information Available: ^{13}C NMR spectra of asmarines C and D. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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